Making Heat-Shock Competent Cells

Overview :

This protocol is generously provided by the lab.
We use DH5α *E. coli* strains.

Materials :

- **2xTY media: 1 L**

Component	Volume & Mass	Procedure
Bactotryptone	16 g	1) Adjust volume to 1 L 2) Sterilize by autoclave
Yeast Extract	10 g	
NaCl	5 g	

- **SOB: 1 L**

Final [c]	Component	Volume & Mass	Procedure
2 %	Bactotryptone	20 g	1) Add NaOH until pH is 6.7, adjust volume to 1 L 2) Sterilize by autoclave
0,5 %	Yeast Extract	5 g	
10 mM	NaCl	2 ml (5 M stock)	
2,5 mM	KCl	2.5 ml (1 M stock)	
10 mM	$MgCl_2$	10 ml (1 M stock)	
10 mM	$MgSO_4$	10 ml (1 M stock)	

- **Transformation Broth (TB): 1 L**

Final [c]	Component	Volume & Mass	Procedure
10 mM	Pipes	3.02 g	1) Mix the Pipes, $CaCl_2$, and KCl in 900 mL of millipore water. 2) Add NaOH until pH is 6.7 (Don't worry, dust disappear after the pH is adjusted) 3) Add MnCl2 (see above), stir, adjust volume to 1 L 4) Filter sterilize 5) Store at 4°C
15 mM	$CaCl_2$	2.21 g	
250 mM	KCl	18.64 g	
55 mM	$MnCl_2$	10.89 g	

Procedure :

DAY ONE:
1) Grow 12 mL overnight culture of your favorite strain of *E. coli* in 2XTY (preheat medium at 37°C before inoculation)
2) Make SOB and TB.

DAY TWO:
1) Keep 5 mL of SOB for initial OD.
2) Inoculate 1 L SOB with 12 mL overnight culture.
3) Grow culture at 18°C (this temperature is really important as we see a 10-fold decrease in competency when we grow them at room temperature).

DAY THREE:
1) Grow cells until A_{600} 0.5-0.7.

Subsequent steps should be carried out in the cold room on ice:

2) Put flask on ice for 10 minutes, then spin cells down at 2500xg (3350 RPM)
3) Pour off supernatant
4) Resuspend gently first in 25 ml TB, then add remaining 295 ml (Final 320mL)
5) Leave on ice for 5 minutes
6) Spin down again at 2500xg for 10 minutes
7) Resuspend cells in 40 mL TB
8) Add 3 mL of DMSO dropwise while gently shaking [final DMSO concentration is 7%].
9) Aliquot in 100 µL aliquots (you will need about 450 pre-chilled 0.5 ml tubes).
10) Flash freeze in liquid nitrogen.
11) Store at -80C.

Heat-Shock Transformation of chemically competent bacteria

1) Take competent cells out of -80°C and thaw on ice (approximately 20-30min).

2) Take agar plates (containing the appropriate antibiotic) out of 4°C to warm up to room temperature or place in 37°C incubator.

3) Label one 2.0mL microcentrifuge tube for each transformation and then pre-chill by placing the tubes on ice.

4) Pipet 1 to 5 µL of DNA (usually 10pg to 100ng) into each microcentrifuge tube. For each transformation, use a separate tube.

5) Pipet 50 µL of competent cells into each tube. Flick the tube gently with your finger to mix. Incubate on ice for 30 minutes. Pre-heat waterbath now to 42°C.

6) Heat shock each transformation tube by placing the bottom 1/2 to 2/3 of the tube into a 42°C water bath for 45 seconds. Do not mix. Be careful to keep the lids of the tubes above the water level, and keep the ice close by.

7) Put the tubes back on ice for 5 min. Do not mix. This helps the cells recover.

8) Add 200µl LB+glucose media (20mM final) **without antibiotic** and grow in 37°C shaking incubator at 180 rpm for 60min.

9) Prepare the agar plates during this time: label them, and add sterile glass beads if using beads to spread the mixture.

10) Plate some or all of the transformation onto a 10 cm LB agar plate containing the appropriate antibiotic.

11) **Note:** We recommend that you plate 50 µL on one plate and the rest on a second plate. This gives the best chance of getting single colonies, while allowing you to recover all transformants.

12) Incubate plates overnight at 37°C. Position the plates so the agar side is facing up, and the lid is facing down.

13) Pipette 5 ml of LB media containing the correct concentration of antibiotic into sterile tubes

14) Pick an isolated bacterial colony with a tip and place the tip with bacteria on it into one of the tubes containing LB liquid media.

15) Culture the tubes overnight in an incubated orbital shaker at 37ºC at 180rpm. The next day you can continue with miniprep protocol.